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Status of phosphine resistance in stored grain insect pests in Uttar Pradesh, India

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ABSTRACT

India is one of the largest foodgrain consumers in the world with large-scale reserves of food grains by the government on a long-term basis. Due to the international agreements for phasing out of methyl bromide, the dependence on phosphine is increasing noticeably in stored grain pest management. Changes in the susceptibility of the pest populations need to be monitored on a long-term basis so as to manage development of resistance to phosphine. Through efficient monitoring programmes, the resistance development can be delayed and thus the effectiveness of
phosphine (Ph_3) can be retained. Studies were carried out to evaluate the susceptibility levels of storage insects populations collected from Uttar Pradesh, India. Insects infesting wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.), viz. *Tribolium casteneum* (Herbst)*, Rhyzopertha dominica* (Fabricius) and *Sitophilus oryzae* (L.), were collected from three different godowns along with the information of their storage method –dose, length of exposure period, number of fumigation, length of storage period and sealing methods. The adult insects were bioassayed in the laboratory. The required dose of phosphine generated from aluminium phosphide was applied through the FAO (1975) method. Discrimination doses, viz. low concentration,0.03 mg^{-1}(*T. casteneum and R. dominica*) 0.04 mg L^{-1} (*S.oryzae*) and high concentration, 0.25 mg L^{-1} for all the three species were used with 20 hr of exposure period for all three species in low and high doses except 48 hr in high dose in *R. dominica.* Mortality was recorded after 7 days from the end of exposure period. The correct mortality was calculated as per Abbot's formula. It was observed that out of eleven populations of *T. castaneum*, one populations showed 100% survival at both discriminatory doses whereas three showed >90%.With 13 *R. dominica* populations, two could not show mortality greater than 10% and 90%survival was observed after bioassay. Again when sixteen populations of *S. oryzae* were bioassayed with both discriminatory doses of phosphine, except two more than 90% survival was recorded all populations. Complete mortality could not be observed with any collected population from Uttar Pradesh to confirm the resistance, molecular screening was also performed to evaluate and quantify frequency of resistant alleles in collected populations.

Key words: Insects, Pests, Phosphine, *Rhizopertha dominica, Sitophilus oryzae,* Stored grains, *Tribolium castaneum*

The food grain production in India is about 250 million tonne with rice and wheat account for a bulk of 170 million tonne and about 30 % of the grains are stored in gunny sack in bulk storage godowns. The stored grains are disinfested from stored grain pests by fumigation with phosphine as in most parts of the world (Rajendran, 1992).

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Resistance to phosphine in stored product pests is a global phenomenon as revealed by widespread reports of resistance in several stored grain pests in countries including Australia, Brasil, Bangladesh, China, Malyasia, Pakistan and Thailand (Daglish and Collins, 1999; Sartori et al., 1990; Mills, 1983; Rahim et al., 2004; Taylor, 1986, Jittanun and Chongrattanameteekul, 2014).Control failures of phosphine fumigation in India had earlier been reported (Rajendran, 1992) and a systematic survey on different

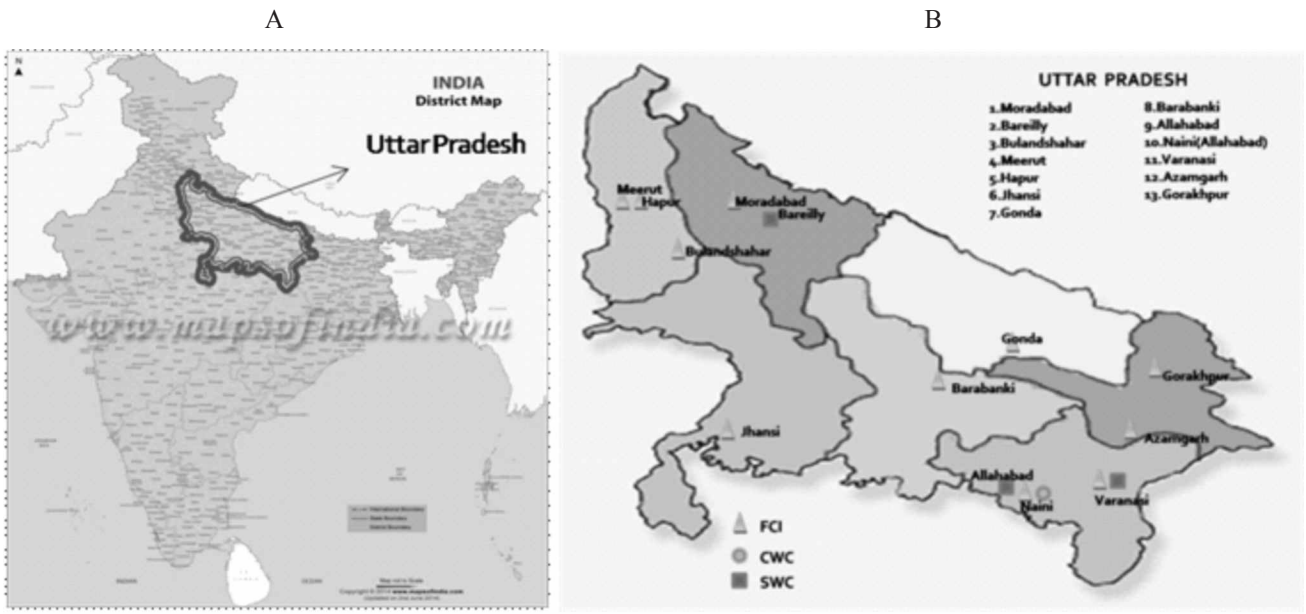

Fig. 1. A, Map showing the sampling area of Uttar Pradesh state, India; B, Sampling locations in the Uttar Pradesh

parts of the country revealed the development of high level of resistance to phosphine in lesser grain borer, *Rhizopertha dominica*, red flour beetle, *Tribolium castaneum* and rice weevil, *Sitophilus oryzae* in India (Rajendran, 1999).

Uttar Pradesh , a major food grain producing state in India which accounts for about one- thirds of the wheat produced in the country and it has a storage capacity of about 47.0 lakh tonne. The present study attempts to document the susceptibility levels of major stored product pests infesting wheat and rice, viz. *Tribolium casteneum, Rhyzopertha dominica* and *Sitophilus oryzae,* collected from bulk storage godowns in Uttar Pradesh state of India.

MATERIALS AND METHODS

Sample collection

Samples of wheat grains were collected from bulk storage wheat godowns in Uttar Pradesh of India during 2012-13 (Fig 1 A,B). The grain samples were collected from godowns managed by three different agencies along with the information of their storage method –dose, length of exposure period, number of fumigation, length of storage period and sealing methods. The adult insects taken from wheat samples were bioassayed in the laboratory.Insect pests from these wheat samples that were then used for this research were thered flour beetle, *Tribolium casteneum,* lesser grain borer, *Rhyzopertha dominica* and rice weevil, *Sitophilus oryzae*.

The insect samples were maintained on wheat flour/wheat grains at constant regimes of 30ºC and 55% r.h. and used for bioassay within 3 to18 months.

Bioassays

The bioassays were conducted at the Stored Product Insect Laboratory of the Division of Entomology, Indian Agricultural Research Institute, New Delhi, India. The required dose of phosphine generatedbydissolving commercially available aluminium phosphide tablets (Celphos® United Phosphoruous Industries, India) in 5% sulphuric acid solution and its concentration was determined by gaschromatography (Nucon-5765) using a thermal conductivity detector (TCD) with Nitrogen (N_2) as the standard.

Phenotypic resistance levels were determined for progeny of adults of *T.castaneum* using a modified FAO method (FAO, 1975). Response of field strains tophosphine were examined by phosphine fumigation at discriminating doses of 0.03 mg^{-1}(for *T.casteneum* and *R.dominica*) and 0.04 mg $L^{-1}$for *S.oryzae.* A high concentration of 0.25 mg $L^{-1}$was used to assay for strong resistance for all the three species.An exposure time of 20 hr was used period for all three species in low and high doses except 48 h was used in *R. dominica* at the high doses.Each assay was replicated thrice and 20 to 50 insects were used in each replicate. After fumigation, mortality wasassessed following a recovery period of seven days in whole wheat flour/ wheat grains at 25±1°C and 55±5% r.h. The correct mortality was calculated as per Abbot's formula.

RESULTS AND DISCUSSION

All the field populations tested of *T. castaneum, R. dominica* and *S. oryzae* exhibited strong resistance to

Table 1. Per cent resistance to phosphine in *T. castaneum* collected from Uttar Pradesh at low and high discrimination doses

Category of per cent resistance	Low concentration (0.03 mg L^{-1})		High concentration (0.25 mg L^{-1})	
	Locations	Per cent resistant (Mean ± SE)	Locations	Per cent resistant (Mean ± SE)
0 – 25%			Gorakhpur	13.33±8.81
25 – 50%			-	-
50 – 75%			Barabanki I	70±5.77
			Allahabad I	70±5.77
75 – 100%	Allahabad I	88.33±1.66	Moradabad	80±5.77
	Meerut	89.34±1.33	Allahabad II	86.66±1.66
	Gorakhpur	90±5.77	Meerut	86.66±1.66
	Varanasi	93.6 ±6.66	Gonda I	90±5.77
	Jhansi	94.66±1.33	Jhansi	91.66±1.66
	Allahabad II	96.6 0±3.33	Gonda II	93.3 ±6.66
	Gonda I	96.66±3.33	Varanasi	93.3 ±6.66
	Moradabad	96.66±3.33	Barabanki II	100±0
	Barabanki I	100±0		
	Barabanki II	100±0		
	Gonda II	100±0		

phosphine as beetles from each collection site survived the discriminating dose for strong resistance (0.25 mgL^{-1}). Bioassays with discriminating doses showed differences in per cent resistance of populations to phosphine fumigation. Of the 11 populations of *T. castaneum* showing strong resistance to phosphine, eight were showing greater than 75% resistance. In the case of *S. oryzae*, 15 field strains were showing varying levels of resistance at discriminating doses of low and high concentrations with 12 strains showing 50% or higher resistance at low dose while nine strains were showing 50% or higher resistance at the high dose. Among the fourteen field strains of *R. dominica,* eight were showing 50 and or higher resistance to phosphine at low dose while at high dose, only five strains were showing 50% or higher resistance to phosphine (Table 1).

Responses of field populations of the three stored product insect species to discriminating doses are mentioned in Tables 2 to 4. Analysis of the per cent resistance values revealed that strong resistance to phosphine is noticed in all the three stored product insects tested at locations, viz. Barabanki, Jhansi and Gonda, while at Gorakhpur and Allahabad locations, strong resistance to phosphine is selected in *T. castaneum* and *S. oryzae*. At bulk storage godowns of Varanasi and Meerut, strong resistance to phosphine is detected in *T. castaneum* and *R. dominica* populations, whereas, populations of *R. dominica* and *S. oryzae* from Bareilly have selected for strong resistance to phosphine.

Phosphine resistance in *Trogoderma granarium* was first detected in India in 1971 (Bora and Chahal, 1979) and subsequent surveys had shown development of resistance to phosphine in major stored grain insects pests including *T. castaneum*, *Sitophilus oryzae*, *R. dominica*, *Oryzaephilus surinamensis, Cryptolestes ferrugineus* and *L. sericorne* (Rajendran,1992; Rajendran et al., 1994). The present study displays the level of resistance to phosphine in contemporary field populations of select stored grain insect pests such as *T. castaneum, R. dominica* and *S. oryzae* collected from a major food grain producing state i.e., Uttar Pradesh. High level of resistance to phosphine is noticed especially in *T. castaneum* with eight out of the eleven field populations showing 75-100% resistance when screened with the high discrimination dose of 0.25 mgL^{-1}. An earlier country wide survey in India documented very high level of resistance to phosphine in *R. dominica* in comparison to *T. castaneum* and *S. oryzae* (Rajendran, 1999).

Ever since the first report of resistance development in *T. castaneum* to phosphine (Champ and Dyte, 1976), the resistance levels in *T. castaneum* were increased to 48.1% in developing countries (Taylor and Halliday, 1986). The present study has highlighted the development of high level of resistance to phosphine in *T. castaneum* with the sampling of field populations from a major grain producing state, Uttar Pradesh. Further studies are required to ascertain the levels of

Table 2 Per cent resistance to phosphine in *S. oryzae* collected from Uttar Pradesh at Low and high discrimination doses

Category of per cent resistance	Low concentration (0.03 mg L^{-1})		High concentration (0.25 mg L^{-1})	
	Locations	Per cent resistant (Mean ± SE)	Locations	Per cent resistant (Mean ± SE)
0 – 25%	Azamgarh I	13.33±8.81	Azamgarh I	6.66±6.61
			Bareilly	20±0
	Bareilly	46.66±12.1	Hapur	30±5.77
25 – 50%	Allahabad I	46.66±20	Moradabad	33.33±3.33
			Allahabad I	40±15.27
50 – 75%	Moradabad	53.33±3.33	Azamgarh II	51.22±20.9
	Azamgarh II	65.32±3.96	Allahabad II	56.66±16.66
	Hapur	66.66±18.5	Barabanki I	63.33±20.7
			Gonda	72.4 ±0.56
75 – 100%	Allahabad I	76.66±8.81	Barabanki II	80±5.77
	Barabanki I	83.33±6.66	Gorakhpur	82.8±10.04
	Gonda	89.82±7.98	Bulandshahr	84.71±11.89
	Gorakhpur	91.10 ±4.5	Jhansi	87.76±9.09
	Bulandshahr	93.48±3.19		
	Barabanki II	96.66±3.33		
	Jhansi	98.85±1.15		

Table 3 Per cent resistance to phosphine in *R. dominica* collected from Uttar at Low and high discrimination doses

Category of per cent resistance	Low concentration (0.03 mg L^{-1})		High concentration (0.25 mg L^{-1})	
	Locations	Per cent resistant (Mean ± SE)	Locations	Per cent resistant (Mean ± SE)
0 – 25%	Barabanki I	23.33±18.55	Barabanki I	6.66±3.33
			Gonda	6.66±6.66
			Varanasi I	10±5.77
			Varanasi II	13.33±6.66
			Meerut I	16.66±8.81
25 – 50%	Varanasi I	40±20	Barabanki II	26.66±3.33
	Bareilly I	43.33±21.85	Bareilly I	36.66±16.66
	Gonda	43.33±26.03	Bareilly II	40±5.77
	Barabanki II	46.66±8.81	Bareilly III	40±11.54
	Bareilly II	50±5.77		
50 – 75%	Meerut I	56.66±24.3	Barabanki III	60±15.27
	Barabanki III	60±23.09	Meerut II	73.33±3.33
	Bareilly III	63.33±23.33		
	Varanasi II	66.66±6.66		
75 – 100%	Meerut II	86.6±6.66	Meerut III	80 ±11.54
	Jhansi I	93.3±6.66	Jhansi I	86.6±13.3
	Meerut III	96.66±3.33	Jhansi II	96.66±3.33
	Jhansi II	100±0		

resistance to phosphine in different stored product pests in India.

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REFERENCES

Anonymous (1975) FAO Method No. 16, Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative method for adults of somemajor pest species of stored cereals, with methyl bromide and phosphine. Plant Protection Bulletin FAO **23**(1): 12–25

Borah G, Chahal BS (1979) Development of resistance in *Trogoderma granarium* Everts to phosphine in Punjab. FAO Plant Protection Bulletin **27**: 77–80.

Cao Y, Zhang J, Bengston M (1999) Studies on a quick method to measure resistance of four strains of Tribolium castaneum (Herbst) to phosphine. (In) Zuxun J, Quan L, Yongsheng L, Xianchang T, Lianghua G (Eds) Proceedings of the Seventh International Working Conference on Stored-product Protection. 14–19 October 1998, Beijing, China. Sichuan Publishing House of Science and Technology, Chengdu, China, pp 603–606.

Champ BR, Dyte CE (1976) Report on The FAO Global Survey of Pesticide Susceptibility of Stored Grain Pests. FAO Plant Protection and Production Services, No. 5, FAO Rome, pp 297.

Daglish GJ, Collins PJ (1999) Improving the relevance of assays for phosphine resistance. (In) Zuxun J, Quan L, Yongsheng L, Xianchang T, Lianghua G (Eds) Proceedings of the Seventh International Working Conference on Stored-product Protection. 14–19 October 1998, Beijing, China. Sichuan Publishing House of Science & Technology, Chengdu, China, pp 584–593.

Jittanun C, Chongrattanameteekul W (2014) Phosphine resistance in Thai local strains of *Tribolium castaneum* (Herbst) and their response to synthetic pheromone. Kasetsart Journal (Natural Science) **48**: 9–16.

Mills KA (1983) Resistance to the fumigant hydrogen phosphide m some stored-product species associated with repeated inadequate treatments. Mitteilungen der Deutschen Gesellschaft fur Allgemeine und Angewandte Entomologie, **4**: 98–101.

Rahim M, Faridah ME, Rasali M (2004) Current status of phosphine resistance in stored grain insects in Malaysia. Journal of Tropical Agriculture and Food Science **32**(1): 101–107

Rajendran S (1992) Insecticide and fumigant resistance of stored product insects in India. Bulletin of Grain Technology **30**: 164–169.

Rajendran S (1999) Phosphine resistance in stored grain insect pests in India. (In) Zuxun J, Quan L, Yongsheng L, Xianchang T, Lianghua G (Eds) Proceedings of the Seventh International Working Conference on Stored-product Protection. 14–19 october 1998, Beijing, China. Sichuan Publishing House of Science and Technology, Chengdu China, pp 635–641.

Sartori MR, Pacheco IA, Vilar RM (1990) Resistance to phosphine in stored grain insects in Brazil. (In) Fleurrat-Lessard F, Ducom P (Eds) 5th International Working Conference on Stored Product Protection. INRA/SDPV, Bordeaux. pp1041-1050.

Taylor RWD (1986) Response of field strains of some insect pests of stored products. (In) Proceedings GASGA Seminar on Fumigation Technology in Developing Countries, Tropical Development Research Institute, London pp 132–140.

Taylor RWD, Halliday D (1986) The geographical spread of resistance to phosphine by coleopterous pests of stored products. (In) Proceedings of the Crop Protection Conference, Pest and Diseases, 17–20 November 1986 Brighton, Metropole, England, pp 607–613.